REMARKS

Claims 2, 7-8, 13-14, 16-19 and 24-25 are pending in the application. The amendments to the claims are made to merely further clarify the presently claimed invention. No new matter has been introduced. Entry of the above revised claims is respectfully requested.

Rejection Under 35 U.S.C. §112, second paragraph

Claims 1, 2, 7, 8, 13, 14, 16-17, 19, 20, 24 and 25 have been rejected under 35 U.S.C. §112, second paragraph, as being indefinite. Applicants traverse this rejection. Reconsideration and withdrawal thereof are respectfully requested. It is believed that this rejection has been overcome by the amendments to the present claims. However, with regard to claim 24, Applicants assert that antecedent basis exists for "adenovirus" in claim 8, from which claim 24 depends.

Rejection Under 35 U.S.C. §112, fourth paragraph

Claim 20 has been rejected under 35 U.S.C. §112, fourth paragraph as it does not further limit the base claims. Applicants traverse this rejection. Reconsideration and withdrawal thereof are respectfully requested.

Claim 20 has been canceled. Therefore, this rejection has been overcome.

Rejection Under 35 U.S.C. §112, first paragraph

Claims 2, 7, 8, 13, 14, 16-20, 24 and 25 have been rejected under 35 U.S.C. §112, first paragraph because of lack of enablement in the specification. Applicants traverse this rejection. Reconsideration and withdrawal thereof are respectfully requested.

The Examiner states in the Office action of September 28, 2011 at page 5 as follows:

> Claims *2, 7, 8, 13, 14, 16-20, 24 and 25* stand rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling a method of treating solid tumors by administering and expression of a nucleic acid molecule encoding the human apolipoprotein (a) kringle KIV9-KIV10-KV (LK68) or KV (LK8) wherein said nucleic acid molecule is carried in a plasmid or AAV or an Adenoviral expression and wherein said vector is administered by a direct injection to the site of tumor, does not enable any route or mode of administration. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

Comparing the Examiner's comments above and the scope of the present claim 18, the Examiner appears to take issue with "intramuscularly injecting" language recited in present claim 18. However, support for this language appears throughout the application. The Examiner's attention is directed to Figures 14, 15, 16, and 18 as well as paragraphs [0050]-[0053] in the U.S. application publication no. 2007/0031379, which explicitly disclose intramuscular injection of the various gene carriers. Therefore, Applicants fail to understand how the present application does not provide enabling claims directed to intramuscular injection of the gene carriers.

Aside from the "intramuscular injection" language, the present claim 18 comports with Examiner's indication of enabled subject matter. Therefore, the present application provides full support and enablement for the presently claimed invention. Accordingly, withdrawal of this rejection is respectfully requested.

Rejection Under 35 U.S.C. §103, Over Chang (WO 01/19868 A1) In View of Trieu (1999, Biochem. Biophys. Res. Comm. 257:714-718) and Kikuchi (2002, Blood 100:3950-3959)

Claims 2, 7, 8, 13, 14, 16-20, 24 and 25 have been rejected under 35 U.S.C. §103(a) as being obvious over Chang in view of Trieu and Kikuchi. Applicants traverse this rejection. Reconsideration and withdrawal thereof are respectfully requested. Claim 20 has been canceled. Claims 2, 8, 13, 14 and 18 have been amended.

Chang

Chang discloses LK8 and LK68 proteins. However, Chang fails to disclose a genetic construct for gene therapy for these proteins.

Trieu

Trieu discloses that a nearly full-size apo(a) protein (18 kringle IV repeats and 1 kringle V) expressed in a transgenic mouse delays tumor growth.

However, a much shorter version comprised of 6 kringle IV and 1 kringle V (termed Ha6) does not suppress tumor growth (Figure 1). At page 715, right column, Trieu states:

> This observation provides unprecedented evidence that a large number of kringle IV repeats is necessary for the biologic activity of apo(a) as an inhibitor of tumor angiogenesis and growth; . . . (emphasis added)

At page 715, right column, Trieu states:

> "thus, the observed lack of anti-tumor effects with this truncated apo(a) protein [Ha6] may be due to its inability to interact with tumor blood vessels.".

In the paragraph bridging pages 716 and 717, Trieu explains this "inability to interact with tumor blood vessels," as follows:

> . . . however, it is of interest that only full-length apo(a) localized to tumor micro-vessels. The fact that truncated apo(a) did not localize to tumor micro-vessels suggests that the missing kringle IV domains are necessary for the binding of apo(a) to tumor micro-vessels to exert its anti-angiogenic effects. (emphasis added)

Trieu fails to disclose or suggest making a gene construct that includes a gene encoding short-length kringle domain such as encoding LK8 or LK68 as in the presently claimed invention.

Kikuchi

Kikuchi discloses a vector comprising the NK4 full length gene, which includes NH_2 terminal hairpin domain and four (4) subsequent kringle domains of hepatocyte growth factor. Kikuchi discloses making AdNK4, which is a replication deficient adenovirus carrying NK4 cDNA. The vector was injected into various cancer model mice. Kikuchi discloses that administering protein form of NK4 inhibits tumor vascularization and induces apoptosis and necrosis of tumor cells. However, AdNK4 single therapy has virtually no therapeutic effect in the murine allograft tumor model. Kikuchi further discloses that tumor size is reduced when

AdNK4 construct is injected in combination with T-lymphocyte stimulating dendritic cells (See Fig. 5 of Kikuchi).

Kikuchi fails to disclose or suggest making a gene construct that includes a gene encoding short-length kringle domains such as encoding LK8 or LK68 as in the presently claimed invention.

Distinctions of the Presently Claimed Invention Over the Cited References

The Examiner has failed to establish *prima facie* obviousness of the presently claimed invention. The Examiner is reminded of the standards for establishing obviousness.

> To reach a proper determination under 35 U.S.C. 103, the examiner must step backward in time and into the shoes worn by the hypothetical "person of ordinary skill in the art" when the invention was unknown and just before it was made. In view of all factual information, the examiner must then make a determination whether the claimed invention "as a whole" would have been obvious at that time to that person. Knowledge of applicant's disclosure must be put aside in reaching this determination, yet kept in mind in order to determine the "differences," conduct the search and evaluate the "subject matter as a whole" of the invention. The tendency to resort to "hindsight" based upon applicant"s disclosure is often difficult to avoid due to the very nature of the examination process. However, impermissible hindsight must be avoided and the legal conclusion must be reached on the basis of the facts gleaned from the prior art. (MPEP 2142)
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> When an applicant submits evidence, whether in the specification as originally filed or in reply to a rejection, the examiner must reconsider the patentability of the claimed invention. The decision on patentability must be made based upon consideration of all the evidence, including the evidence submitted by the examiner and the evidence submitted by the applicant. A decision to make or maintain a rejection in the face of all the evidence must show that it was based on the totality of the evidence. Facts established by rebuttal evidence must be evaluated along with the facts on which the conclusion of obviousness was reached, not against the conclusion itself. *In re Eli Lilly & Co.*, 902 F.2d 943, 14 USPQ2d 1741 (Fed. Cir. 1990). (MPEP 2142)

The instant rejection is based upon the contention that it would have been obvious to one of skill in the art to place sequences encoding LK68 and LK8, as reported by Chang into the vector constructs of Trieu, Kikuchi, or Kuo (cited previously) with the expectation of using them as gene carriers to increase the efficacy of tumor gene therapy. The Examiner appears to believe that therapeutic use of vectors is routine in the art.

Chang discloses anti-angiogenic effects of LK8 and LK68. However, Chang fails to disclose a genetic construct for gene therapy for these proteins. Trieu is cited to remedy this

deficiency by pointing to successful anti-angiogenic effects of full length apo(a) in transgenic mice. However, Trieu discloses that a gene vector including an apo(a) fragment of 6 kringle IV and 1 kringle V does not suppress tumor growth. Trieu cites the necessity for a gene fragment of apo(a) that is longer than this failed fragment. Trieu affirmatively guides the person of ordinary skill in the art to create anti-angiogenic fragments of apo(a) that are longer than 6 kringle IV and 1 kringle V. Trieu bluntly states that "the missing kringle IV domains are necessary for the binding of apo(a) to tumor micro-vessels to exert its anti-angiogenic effects." (see paragraph bridging pages 716 and 717).

In order to further remedy the deficiencies in Chang and Trieu, the Examiner cites Kikuchi and states as follows at page 9 in the Office action of April 13, 2009:

> However at the time of invention Kikuchi teaches tumor therapy with using kringle-4 containing fragments. Kikuchi teaches gene therapy of tumor using Adenovirus vector containing gene encoding NK4 kringles, short Kringle-4 containing fragments (entire article; abstract)

The Examiner further applies Kikuchi to the claims by stating the following in the paragraph bridging pages 9 and 10 in the Office action of April 13, 2009:

> Thus it would have been obvious to one of skill in the art to try gene therapeutic approach that would parallel the success of polypeptide therapy using LK8 and LK68 apo(a) protein kringle fragments as taught by Chang and further substitute the same for full length apo(a) gene therapeutic vector construct of Trieu or short kringle coding sequences in viral vectors described by Kiuchi or other gene therapy vectors taught by Kuo and prepare and use a composition to treat a solid tumor in an animal subject. One

The Examiner states that Kikuchi discloses gene therapy using Adenovirus vector containing "short Kringle-4 containing fragment" and that the claimed invention directed to gene carrier comprising gene encoding LK8 or LK68 (which are very short fragments of apo(a)) is obvious because Kikuchi discloses a gene therapy vector comprising a "short Kringle-4 containing fragment".

The references fail to be combinable with each other

The references fail to be combinable with each other for the following reasons. First of all, Kikuchi discloses using NK4 protein, which is not an apo(a) protein of Chang and Trieu references. Therefore, this raises an immediate issue of combinability of the Kikuchi reference with the Chang and Trieu references. A person of skill in the art making an apo(a) fragment would not look to a reference disclosing the manipulation of NK4 protein for any guidance. Therefore, these references fail to be combinable with one another.

Secondly, the Chang reference discloses a short LK8 (1 kringle) and LK68 (3 kringles) kringle domain peptide fragments. Trieu discloses the "necessity" of using genes encoding large fragments of apo(a) having more than 6 kringle IV's. By the very nature of these disclosures, Chang and Trieu fail to be compatible or complementary with each other as they disclose opposite results. And Kikuchi discloses an entirely different protein. Accordingly, the presently claimed invention is not obvious over the cited references.

Even if combined, the references fail to arrive at the presently claimed invention

The deficiencies of Chang and Trieu references are discussed above. However, Applicants fail to understand the Examiner's characterization of the Kikuchi reference and its relevance to the presently claimed invention. As far as Applicants can see, Kikuchi discloses making a vector that includes full-length NK4. Kikuchi fails to disclose or suggest breaking up the full-length NK4 protein in any way. Kikuchi fails to disclose or suggest inserting any single or short kringle-4 domain into a gene vector.

NK4 protein is a variant of Hepatocyte Growth Factor (HGF) protein. Contrary to HGF, NK4 full length version has anti-angiogenic activity. The NK4 gene cloned into the gene vector recited in Kikuchi is not a single kringle domain but rather a protein similar to the full length HGF comprising N-terminal hairpin loop and 4 kringle domains. Its molecular weight is 67 kD while that of full-length HGF alpha chain is 69 kD.

Kikuchi discloses that intratumoral injection of Ade-NK4 (Adenovirus/full-length NK4 construct) has little effect on the size of the tumor. Kikuchi discloses that tumor size is reduced when Ade-NK4 construct is injected in combination with T-lymphocyte stimulating dendritic

cells. Therefore, the point of the Kikuchi reference is that dendritic cells contribute to anti-angiogenic effect of an otherwise weakly effective Ade-NK4 gene construct.

Moreover, Merkulova-Rainon et al., J. Biol. Chem, 278(39): 37400–37408, 2003 (Exhibit A, submitted with the Amendment filed October 2, 2009, provided herewith for the Examiner's convenience) discloses that N-terminal hairpin loop rather than any 4 kringle domains is important for anticancer activity of HGF (Abstract).

Given that Chang fails to disclose or suggest a gene therapy vector housing a gene encoding LK8 or LK68, and Trieu guides the skilled artisan to make a vector incorporating only genes encoding large fragments of apo(a), and further Kikuchi discloses an unrelated NK4 gene having 4 kringle domains, and which further fails to disclose or suggest constructing a gene therapy vector including a small fragment of apo(a) gene such as LK8 (1 kringle V) or LK68 (2 kringle IVs and 1 kringle V), the combination of the references fails to arrive at the claimed invention. Therefore, the presently claimed invention is not obvious over the cited references.

Additional considerations

Teaching away of the Kuo reference

In the various communications with the Patent Office, Applicants particular disagree with one line of reasoning given by the Examiner. When a reference that "teaches away" from the claimed invention is cited to the Examiner, the Examiner argues that the reference is not a "teaching away" but rather the reference would further motivate the person of skill in the art to overcome the deficiency in the reference to make the claimed invention.

Examples of this situation is found with the Examiner's citation of Kuo (see above) and the Examiner's characterization of Trieu. Discussing Kuo first, Applicants had initially brought the Kuo reference to the attention of the Examiner in the Amendment filed on December 7, 2007, to show that Kuo reports that an adenovirus based vector system used to express endostatin or angiostatin demonstrates little or no inhibition of tumors in an animal model despite potent antitumor effects of endostatin and angiostatin when the protein is delivered directly.

The Examiner dismissed the Kuo reference that shows a failed gene therapy attempt and instead cited this reference against the presently claimed invention as exemplifying various gene

therapy vectors that could be used in making the gene construct of the presently claimed invention. In the interview held on June 26, 2008, when confronted with the disclosure in Kuo that employing these vectors in expressing endostatin and angiostatin fails to result in any effective antitumor effect, the Examiner indicated that Kuo actually provides motivation to a person of skill in the art to insert the gene encoding LK8 and LK68 in a gene therapy vector instead of endostatin and angiostatin because LK8 and LK68 were shown by Chang to have anti-angiogenic activity at the peptide level.

However, the Examiner is reminded that endostatin and angiostatin also has anti-angiogenic activity when the protein is administered. The surprising finding in Kuo is that the gene therapy vector containing the gene encoding endostatin and angiostatin unexpectedly does not exhibit anti-tumor effects even though the genes are expressed and are present in the serum. So, why would a skilled artisan expect a vector construct harboring gene encoding LK8 or LK68 to have any different effect than portrayed in Kuo for the failed endostatin and angiostatin genes? Where is the reasonable expectation of efficacious success of a gene therapy construct by including a gene encoding LK8 or LK68? The Examiner's suggestion that the person of skill in the art would have been motivated to insert LK8 or LK68 gene into Kuo's failed gene therapy vector with a reasonable expectation of success is on its face an improper application of the standards of establishing obviousness under 35 U.S.C. 103(a).

If LK8 or LK68 genes were indeed placed in the vectors described in Kuo, would a person of skill in the art reasonably expect to obtain effective tumor suppressing activity? The answer must a resounding "No", because Kuo already demonstrates that these vectors are not successful in suppressing tumor growth even though the endostatin and angiostatin proteins that are expressed from the gene therapy vector are observed in the serum of the infected mice (page 4609, left column). The Examiner is reminded that both endostatin and angiostatin possess kringle domains. The gene therapy vectors that are cited in Kuo may work for some genes, but certainly does not work for endostatin and angiostatin. The Examiner's decision to simply waive off the Kuo reference is contrary to law. The Examiner is required to consider the totality of the evidence presented in considering obviousness of the presently claimed invention.

The only expectation of success is found in the instant application, where there is demonstration of successful use of nucleic acids to express LK68 and LK8. With such success,

no more than routine and repetitive experimentation is needed to practice the claimed methods.

Teaching away of the Trieu reference

When it was pointed out to the Examiner that the Trieu reference taught away from the claimed invention directed to gene therapeutic expression of LK8 (1 kringle) or LK68 (3 kringles), which are short fragments of apo(a), the Examiner simply waived off this argument without an explanation and again stated that Trieu is a disclosure that would motivate a person of skill in the art to actually insert LK8 or LK68 gene in a gene therapeutic vector. Applicants fail to see how a reference that expressly teaches that in order to make an anti-angiogenic fragment from apo(a), it is "necessary" to make it longer than a 7 kringle fragments, could somehow be used to obviate the claimed invention directed to a fragment of apo(a) that is much shorter than the Trieu fragment. Trieu says that it does not expect any fragment of apo(a) that has 7 or less kringle domains to have anti-angiogenic activity. The presently claimed invention has 1 kringle fragment (LK8) and 3 kringle fragments (LK68).

The Examiner argues that in spite of this negative teaching of Trieu, since Chang does disclose that small LK8 and LK68 protein fragments do have anti-angiogenic activity, a person of skill in the art reviewing both Chang and Trieu would be motivated to include the gene encoding LK8 or LK68 into the vector of Trieu, even if Trieu in effect expressly discloses that a gene encoding LK8 or LK68 would not be expected to be effective to suppress tumor growth.

The Examiner has failed to consider the totality of the evidence. The Examiner chose to focus on the protein effects of Chang, but failed to consider the deeper ramifications of kringle expression through gene therapeutic vehicles. Kuo is a case in point as discussed above regarding the disparity between protein administration activity and gene therapy activity.

When Chang and Trieu are combined with the teachings of Kuo, a person of ordinary skill in the art would come to the conclusion that even though Chang discloses LK8 and LK68 peptides as having anti-angiogenic activity, a level of reasonable expectation of success would not be achieved within gene therapy context based on at least two considerations: (1) Kuo states that there is a disparity between protein administration activity levels, and gene therapy approach; and (2) Trieu states that anti-angiogenic fragment of apo(a) to be effective in a gene therapeutic setting must possess more than 6 kringle IV domains. A person skilled in the art,

armed with the knowledge that gene therapeutic effects are not readily predictable from protein administration effects would have doubts of the likelihood of success that LK8 or LK68 would be effective in a gene therapy protocol of Trieu. Therefore, given the totality of the evidence, the presently claimed invention is not obvious over the cited references.

State of the art at the time of the invention regarding angiogenic or anti-angiogenic activity of apo(a) was confusing

As discussed above, the apo(a) art as well as proteins that possess kringle regions, exhibit unusual and sometimes inconsistent results. This results in an unsettledness and predictability of the activities of: (i) apo(a) protein, (ii) apo(a) fragments, and (iii) genes encoding apo(a) or apo(a) fragments. The activities of these molecules often do not correlate with each other. Some of the inconsistent activities are known in the art. A brief discussion follows:

Trieu discloses that the full length apo(a) is an anti-angiogenic protein. Other references disclose that full-length apo(a) has angiogenic activity.

Yano et al., "Stimulatory effects of lipoprotein(a) and low-density lipoprotein on human umbilical vein endothelial cell migration and proliferation are partially mediated by fibroblast growth factor-2", Biochim. Biophys. Acta 1998;1393:26–34 (Exhibit B, submitted with the Amendment filed October 2, 2009, provided herewith for the Examiner's convenience) discloses the opposite effect from Trieu. Yano discloses that full length apo(a) is an angiogenic protein.

In Liu et al., "Apolipoprotein(a) stimulates vascular endothelial cell growth and migration and signals through integrin αVβ3", Biochem. J. 2009;418:325–336 (Exhibit C, submitted with the Amendment filed October 2, 2009, provided herewith for the Examiner's convenience), the authors identified a stimulatory effect of apo(a) and Lp(a), but not LDL, on HUVEC proliferation and migration, which suggests a potential role for apo(a) in regulating important physiological/pathological events including angiogenesis, tumor invasion and metastasis, and wound healing. Liu discloses that apo(a) stimulates HUVEC proliferation and migration, and furthermore, induces angiogenesis, tumor invasion and metastasis, and wound healing. It also demonstrates that the LBS in the KIV10 and in the KV, which also constitute

LK8 and/or LK68, are essential for these apo(a) activities. Lieu discloses that apo(a) is a stimulator rather than inhibitor, of angiogenesis.

Lou (Exp. Mol. Pathol. 65(2): 53-63, 1998) (Exhibit D, Abstract only, submitted with the Amendment filed October 2, 2009, provided herewith for the Examiner's convenience) also reports that apo(a) is a stimulator of angiogenesis.

Given the above divergent results obtained regarding the activity of full length apo(a), a person of skill in the art would be confused as to the role of apo(a) in angiogenesis, and further would be dissuaded from making any gene therapeutic constructs including these genes encoding the various fragments. Indeed, the Chang reference may be confusing to the skilled artisan given the background and dislosures of apo(a) above, especially in view of Trieu, which discloses that a fragment as close to the full length must be used for anti-angiogenesis activity, which is a directly opposite teaching from Chang. Therefore, a person of skill in the art would be dissuaded from making a gene therapeutic construct given the overall uncertainty associated with the function of apo(a).

Gene therapy and protein therapy do not yield similar results

As discussed above, gene therapeutic effects are not readily predictable from protein administration effects. The following is a short discussion of the state of art.

The Kuo reference discussed above discloses that whereas angiostatin, endostatin, and neuropilin has good effects when protein is administered, they are significantly less effective when the subject is transfected with adenovirus harboring the genes encoding these proteins. Kuo states the following in the abstract, "these data underscore the need for comparative analyses of different therapeutic approaches that target tumor angiogenesis.

Joseph (Cancer Gene Therapy, 10: 859–866, 2003) (Exhibit E, submitted with the Amendment filed October 2, 2009, provided herewith for the Examiner's convenience) also discloses that in the case of angiostatin, whereas the protein version of Kringle 1-3 had anti-angiogenic effects, the gene therapeutic construct of Kringle 1-3 resulted in no delay in tumor growth.

Given this divergent angiogenic/anti-angiogenic results obtained with administering the protein as opposed to the gene construct encoding these proteins that comprise kringle domains,

it must be questioned whether a successful gene therapeutic effect is predictable from the protein administration effects in this field. Applicants submit that there is enough variability in the field to indicate that it would be unpredictable to automatically assume that a gene therapeutic construct would successfully follow in the activity path as administering the protein alone.

Unexpectedly superior activity of LK8 and LK68 expressed from gene construct over the Chang peptides

The Examiner's attention is again directed to the 132 Declaration of Dr. Eui-Cheol Jo, an inventor in the present application, (filed with the Amendment of October 2, 2009, provided herewith for the Examiner's convenience) showing unexpectedly superior cell migration inhibiting activity of the LK8 and LK68 protein expressed from mammalian gene constructs harboring nucleic acid encoding LK8 or LK68 in mammalian cells. Adenovirus and AAV expressed LK8 and LK68 peptide prevented migration of endothelial cells at an unexpectedly high level compared with LK8 and LK68 peptides produced from *E. coli* as in Chang.

While it is well known that proteins produced from mammalian cells tend to have higher activity than those produced from *E. coli*, usually due to glycosylation and so forth found in proteins produced from mammalian cells, Dr. Jo provides evidence in the Declaration that indeed a 100 fold increase in activity was obtained and that this increase was not due solely to conventionally understood glycosylation of the proteins.

The unexplained and remarkably superior activities of LK8 and LK68 expressed from a gene construct in mammalian cells may explain why the therapeutically active levels of the inventive gene expressed LK8 and LK68 is much higher than at protein administration levels. Clearly, LK68 and LK8 peptides produced in mammals are fundamentally different in size and activity from *E. coli* produced LK8 and LK68 peptides such as disclosed in Chang, but such high activity could not have been predicted even with the general knowledge in the art that mammalian cell processed proteins have a different activity from *E. coli* produced cells. Quite simply, a 100 fold increase in activity of the gene construct of LK8 and LK68 over protein administration constitutes an unexpectedly superior result. But this result is made even more surprising considering the fact that Kuo had indicated that gene therapy results would not likely

be successful even if the protein administration may be effective, and that Trieu discloses that an apo(a) fragment of less than 6 kringle domains would not have anti-angiogenic activity. Accordingly, the presently claimed invention is not obvious over the cited references.

Summary of Some of the Distinctions of the Presently Claimed Invention Over the Cited References

1) The size of the Chang LK8 and LK68 and the gene product produced from the inventive gene construct indicates a basis for the unexpectedly superior anti-cancer and anti-metastatic effects of the inventive gene construct.

Chang discloses the LK68 or LK8 protein sequence. However, the size of the protein which is actually generated *in vivo* by using the presently claimed vectors is different, partly due to glycosylation of the produced protein. This may account for the unusually high anti-cancer and anti-metastatic effect of using the gene versus the protein compared with protein therapeutics of Chang.

To be more specific, the size of recombinant LK8 protein disclosed in Chang is approximately 10kDa. (See Reference 1 (submitted with the Amendment filed September 7, 2010, provided herewith for the Examiner's convenience) - see 'Results and Discussion' and Fig. 1 on page 536, left column, Biochem and Biophys Res Commun. 2004 Jan 16;313(3):534-40). On the other hand, the size of the corresponding gene product of the presently claimed invention is 15kDa. (Reference 2 (submitted with the Amendment filed September 7, 2010, provided herewith for the Examiner's convenience) - please see page 1065, 'RESULTS: rAAV-Mediated Expression of LKs In Vitro' Hepatology 2006;43:1063-1073).

The size of LK68 recombinant protein is 37kDa (See Fig.1 of Chang, and Reference 3 - (submitted with the Amendment filed September 7, 2010, provided herewith for the Examiner's convenience) 'RESULTS' and 'Fig. 1' on page 29002, left Column, JBC 2003;278:29000-29008), but the size of the gene product of the presently claimed invention is 55kDa. (See Reference 2, page 1065).

It is assumed that the differences in size is caused at least in part by glycosylation of proteins generated *in vivo*, and it is known that the activity of this gene product is much improved compared with Chang's recombinant protein (See Reference 4, (submitted with the

Amendment filed September 7, 2010, provided herewith for the Examiner's convenience) Fig. 1 and Fig. 2, Molecular Therapy, 2004;9:56-60). Therefore, this large improvement in activity could not have been expected from the recombinant LK68 or LK8 protein of Chang.

Accordingly, the gene therapeutics of the presently claimed invention has unexpectedly superior anti-cancer and anti-metastasic activity compared with the proteins described in Chang. Moreover, a person skilled in the art could not have predicted whether a gene will undergo any protein modification, including glycosylation, and whether the resultant modification would cause any particular advantage when the gene fragment encoding the amino acid sequence of the LK68 or LK8 protein is administered *in vivo*. So it cannot be said that Chang provides the motivation to make the gene construct of the presently claimed invention. This is particularly relevant to the claimed invention because the gene fragment that is used is not a naturally occurring gene. Rather, LK68 and LK8 are fragments of the naturally occurring protein apolipoprotein(a). And as a result, it would have been further unpredictable to determine whether a resultant gene product modification would have beneficial effects.

2) Prior art shows failed gene therapy even after the recombinant protein showed promising activity, which indicates that the art is unpredictable with regard to gene therapy.

In view of the fact that even if it is known in the art that certain proteins have therapeutic activity, yet gene therapy constructs have shown little or no additional anti-cancer or anti-metastatic benefit and even some constructs exhibit no activity at all, it cannot be concluded that the LK68 or LK8 protein of Chang is predictive of the superior gene therapeutic activity of the presently claimed invention.

For instance, angiostatin (Reference 5 (submitted with the Amendment filed September 7, 2010, provided herewith for the Examiner's convenience) – Cell, 1994 Oct 21;79 (2):315-328, Abstract) and endostatin (Reference 6 (submitted with the Amendment filed September 7, 2010, provided herewith for the Examiner's convenience) – Cell, 1997 Jan 24;88(2):277-285, Abstract) in protein form show anticancer activity in Lewis Lung Carcinoma and T241 Fibrosarcoma. However, when the gene constructs for angiostatin and endostatin are used in gene therapeutics, they show no anticancer or anti-metastasic effect at all. (Reference 7 (submitted with the Amendment filed September 7, 2010, provided herewith for the Examiner's

convenience) - PNAS, 2001;98:4605-4610, and Reference 4 - Molecular Therapy 2004;9:56-60).

3) LK68 or LK8 are proteins with liver-specific biosynthesis, and therefore, activity observed based on non-liver related assays are not predictive of the activity of LK68 and LK8 gene construct.

According to the Background section in the present specification, apolipoprotein (a) is biosynthesized in the liver and shows pharmacological effect at the targeted site after it is circulated in the blood.

When a pharmaceutical composition containing LK68 or LK8 protein of Chang is injected intravenously, it circulates in the blood and shows pharmacological effect, whereas if the gene which expresses the same protein is injected as gene therapeutics or cell therapeutics, the injected part of the genes or cells biosynthesizes the target protein in the liver, which results in a different effect from injected proteins, which result would have been unpredictable due to the different level of modification of the resultant gene product.

4) The present application discloses more advantageous effects of gene therapeutics compared with Chang.

(i) Whereas an anticancer agent containing a protein as an effective ingredient has comparatively short half-life *in vivo* and thus, in order to maintain an anticancer effect, the required amount of protein has to be administered constantly to maintain the level of anticancer protein in tumor cells, the presently claimed invention solves this problem by using gene therapy, which surprisingly results in expressed protein that has unexpectedly superior activity.

(ii) Whereas a problem with using protein therapy is that a huge amount of protein is required, and as a result, facilities and techniques should be equipped for producing proteins, which can be expensive for both patients and producers, the presently claimed invention solves this problem by using gene therapy, which surprisingly results in expressed protein that has unexpectedly superior activity.

iii) Whereas using the Chang recombinant protein carries a risk of bearing bacterial endotoxin caused by protein produced from micro-organisms, the presently claimed invention

solves this problem by using gene therapy, which surprisingly results in expressed protein that has unexpectedly superior activity, and does not carry any microorganism endotoxin.

The presently claimed gene therapy method allows for production of easily sustained concentration level of the anti-cancer protein at the systemic, local or regional tumor with a single administration, with superior effects compared with direct injection of the anticancer protein. As inhibition effect of the LK68 and LK8 genes on the tumor volume is superior to the LK68 or LK8 proteins, the presently claimed gene therapeutics is unexpectedly superior to the use of Chang's LK8 and LK68.

5) LK68 or LK8 gene of the presently claimed invention preserves the genes' original anticancer activity *in vivo* regardless of the characteristics of the gene carrier.

i) Gene therapeutics of the present invention has superior anticancer or anti-metastasic effect due to the expressed protein modifications, including glycosylation made *in vivo* compared with direct injection of LK68 or LK8 protein of Chang.

ii) Inhibition effect of tumor volume is unexpectedly superior using the gene construct of the presently claimed invention. Example 10 of the present application discloses the result of growth inhibiting effect of metastatic cancer through injection of LK68 or LK8 gene. The adeno-associated virus that includes retrovirus LK68 gene carrier (rAAV-LK68) or LK8 gene carrier (rAAV-LK8) was injected in the three parts of hind leg muscle of Balb/c nude mouse to express the LK68 or LK8 *in vivo*. 2 weeks after injection, B16F10 melanoma cell (ATCC, Manassas, VA, USA) was injected through the tail vein. After another 2 weeks, lung of the mouse was extracted and black tumor cell swellings of the surface were counted. LK68 or LK8 had approximately 30~60% inhibiting effect on metastatic cancer. See Figure 13. In this experiment, rAAV-lacZ with pAAV-lacZ (Stratagene, USA) which expresses beta-galactosidase was used as negative control, and rAAV-K13 was used as positive control. Fig 13(a) and Fig 13(b) show that the inhibiting effect of metastatic cancer using control angiostatin (K13) is similar to that of using LK8 or LK68.

However, according to Fig 14, the cancer volume growth inhibiting effect of the control angiostatin (K13) is meager compared with that of the gene therapeutics of the presently claimed invention. That is, the present Fig 14 and Example 11.1 disclose the liver cancer growth

inhibiting effect of LK68 and LK8 gene in the solid liver cancer model using Huh-7 Human hepatocellular carcinoma cell. Here, only saline solution was injected as negative control, rAAV-K13 as positive control. rAAV-GFP and rAAV-lacZ with pAAV-hrGFP (Stratagene, USA) were used to measure the infection efficiency. As a result, the expression of concentration of LK68 remained 50-150 ng/ml, LK8 remained 30-50 ng/ml, and K13 remained 50-100 ng/ml. It also discloses approximately 80-90% of growth inhibiting effect of LK68, LK8 in solid liver cancer. See Fig 14.

Fig. 14(a) shows that cancer volume when rAAV-K13 was used as positive control measured approximately 3500 mm^3 after 30 days of virus (gene therapeutics) injection, which indicates that the growth inhibiting effect of tumor volume is meager even compared with the result of using saline solution (2500 mm^3) as negative control. In contrast, the volume of tumor when rAAV-LK8 was used was approximately 500 mm^3 and for rAAV-LK68 was 250~300 mm^3, showing great growth inhibiting effect of cancer volume for a long period.

In Examples 1 to 3 in the present application, LK68 or LK8 DNA was introduced to pLXSN vector, a retrovirus vector, which was transfected into packaging cell line PT67, producing recombinant virus. By infecting the recombinant virus into CT26, colon cancer cell line, the effect of cancer cell apoptosis and the result of cancer growth inhibiting effect *in vivo* was disclosed. Fig 3a shows 80% tumor growth inhibiting effect of CT-LK68. The excellent tumor growth inhibiting effect was shown in that cancer volume was approximately 1000 mm^3 after 30 days of transplantation of gene carrier into tumor while CT-mock remained 8000 mm^3 and CT-vector as 7000 mm^3.

LK68 or LK8 gene therapeutics has significantly greater effect than what the person with skill in the art can expect from the cited art. No matter what the characteristics of the gene carrier is, it can sustain anticancer activity of the gene *in vivo*.

Examples 4 to 6 in the present application shows the inhibiting effect of cancer metastasis in spleen metastasis model and abdominal cavity metastasis model. The proliferation

process of macrometastasic cancer can be inhibited by making micrometastasic cancer through new blood vessel, which proves the useful value of the present invention as gene therapeutics.

6) The presently claimed gene therapeutics possesses stable treatment effect for a long time *in vivo*, which is not obvious over the cited references.

There are many obstacles to overcome in the field of gene therapeutics based on results from protein therapeutics. One of the most important factors for successful gene therapeutics for anticancer or anti-metastasis is "long and stable expression of the introduced gene" and its "long treatment effect". To achieve this goal, many vector systems have been developed. However, it would not have been predictive as to which vector would be optimal for use as in the claimed invention, as Chang fails to provide or suggest any such particular vector.

To be more specific, apolipoprotein(a) is a protein that specifically biosynthesizes only in the liver. However, Chang fails to disclose or suggest that the gene fragment coding for LK68 or LK8 protein can be combined with any particular vector, which would remain expressed steadily in particular tissue or organ as well as liver tissue.

Chang merely suggests that a gene encoding apolipoprotein(a) having anti-cancer activity in certain tissues may be introduced into a gene carrier for gene therapy using technology available to one of ordinary skill in art. However, it is not predictable from Chang if any of these vectors would function successfully *in vivo* in tissue or organ as gene therapeutics.

Compared to the injection of LK68 or LK8 protein as anticancer agent, the presently claimed gene therapeutics has long and stable effect *in vivo*, compared with Chang and the prior art.

Fig 17 shows that 30% (rAAV-LK8) ~ 40% (rAAV-LK68) of injected group have survived 170 days after the injection of the gene, which means 30% of rAAV-LK8 and 40% of rAAV-LK68 of the injected group completely overcame the solid cancer. This type of result could not have been expected from the Chang disclosure in which LK68 or LK8 protein is directly injected as anticancer agent. Gene therapeutics of the presently claimed invention provides significantly improved effect as seen in Reference 2, page 1063~1073.

Recombinant adeno-associated virus (rAAV-LK68 or rAAV-LK8) significantly inhibits proliferation and migration of HUVECs *in vitro*, and the inhibition rate of tumor was decreased

60~84% in mouse model transplanted from hepatocellular carcinoma cells of Huh-7 and Hep3B. The result of histological and immunochemical tumor shows that a single inoculation of rAAV-LK68 or rAAV-LK8 dramatically inhibits tumor growth, and increases the survival rate of inoculated mice ($P<.05$). 3 of 10 inoculated mice survived without visible cancer and clinical signs after 188 days of inoculation of rAAV-LK68 and rAAV-LK8. This shows superior result, which could not have been predicted from the combination of vector systems of Chang and in the field of gene therapeutics.

7) The Kikuchi reference is not applicable to the presently claimed invention.

Regarding the Kikuchi reference, while the disclosed α-chain of HGF is 69 kD, in Kikuchi, 67kD is NK4 and a fragment with little difference in length compared to α-chain of HGF. The present invention uses LK8 (1 kringle V), and LK68 (2 kringle IV and 1 kringle V), i.e., only partial kringle fragment of huge molecular apo(a). When the amino acid sequences of NK4 and HGF are compared, there is a little difference in that HGF consists of 463 amino acids and NK4 consists of N-terminal 447 amino acids of α-chain of HGF.

Also, there is a difference in construct in that NK4 consists of hairpin domain and four kringle domains in N-terminal, while the presently claimed invention consists of only kringle domain without hairpin domain. Among the sequence of total 447 amino acids of NK4, the hairpin domain consists of about 80~90 amino acids.

In Merkulova-Rainon (Exhibit A, submitted with the Amendment filed October 2, 2009, provided herewith for the Examiner's convenience), only the N-terminal end of HGF has angiogenesis activity while HGF separated from the N-terminal end does not have this activity. Therefore, it can be inferred that anti-cancer activity of HGF is related to the hairpin domain of N-terminal, not the kringle domain.

The technical feature of Kikuchi is that NK4 alone does not have significant anti-cancer activity, but does have it with interaction of NK4 and dendritic cell, which is incompatible with the technical feature of the presently claimed invention.

In particular, Kikuchi (See, 3953-3956 page, Results section) discloses intratumoral injection of AdvNK4 alone into tumor therapy mice model produced by subcutaneously

injecting tumor cells, which results in no effect on growth of tumor and shows no cytotoxic activity of splenocytes, as is the case of mice with no treatment.

Kikuchi also discloses that AdvNK4 alone does not have anti-cancer activity, and has it only by interaction with the subject in which the dendritic cell stimulates T- lymphocyte.

Therefore, to summarize, there is a molecular weight difference in the LK68 and LK8 proteins produced by Chang and the gene therapeutics of the presently claimed invention. The gene product of the presently claimed invention has a superior effect compared with injecting the protein itself as an anticancer agent.

Even if a protein is known to be an anti-cancer or anti-metastatic agent, similar success cannot be automatically assumed for its use in gene therapeutics. Thus, even if Chang discloses that LK68 or LK8 protein has anticancer or anti-metastatic activity, the same level of success could not have been expected with a reasonable expectation with combination of Trieu and Kikuchi. Gene therapy protocol is significantly different from protein therapeutics, therefore, the gene and its protein is not expected to have bioequivalent activity as therapeutics.

The presently claimed invention is not obvious over the cited references for describing gene therapeutics that has long and stable anticancer therapeutic effect, which cannot be envisioned with a reasonable expectation of success from protein anticancer agent of Chang, in combination with Trieu and Kikuchi. Accordingly, the presently claimed invention is not obvious over the cited references.

Applicants' further comment in reply to the Office action of September 28, 2011

The Examiner states at page 12 that as follows:

> The Applicants arguments however found not persuasive because in the first place the art as taught above do not teach away from gene therapy of tumors with sequence encoding kringle domains of Apo(a) protein. Not succeeding in using the gene sequences encoding shorter fragments of kringles of Apo(a) gene as compared to his significant success with the full length Apo(a) gene does not imply that Trieu is teaching away from using shorter fragments of instant invention.

Trieu discloses that a small fragment of Apo(a) gene does not suppress tumor growth. This is at the gene level. This is a teaching away from the presently claimed invention directed to using a small fragment of Apo(a) gene to reduce tumor growth. These are opposite

conclusions. Therefore, it is not understandable how Trieu can be applied to the presently claimed invention. When this prior art reference discloses the opposite effect of the claimed effect, it is proper and fair to conclude that the Trieu reference starkly teaches away from the presently claimed invention.

Chang discloses a fragment of the protein form of Apo(a), which is used in its protein form for anti-angiogenic effects. However, the Chang reference leaves a question regarding whether using the gene form would work at all. It was by no means entirely predictable in the art that gene form of a molecule can work just as well as or at all compared with its protein form. Applicants have provided journal articles regarding this submitted with the Response of October 2, 2009, and asserted again in the Response of November 17, 2010. The Examiner is requested to revisit the various references regarding the state of the art at the time of the invention regarding the role of Apo(a) and the uncertainty with respect to gene therapy from protein effects. In particular, the Examiner's attention is directed to the Joseph reference (Exhibit E, originally submitted with the Amendment filed October 2, 2009, provided herewith for the Examiner's convenience) that discloses that a gene construct with Kringle 1-3 did not result in delay of tumor growth. Therefore, it is not a safe assumption to state that successful gene therapy is automatically predictable from protein results.

The Examiner acknowledges that as follows at page 13:

> However, even though applicant's modification results in great improvement and utility over the prior art, it may still not be patentable if the modification was within the capabilities of one skilled in the art.

The Examiner acknowledges that gene therapy method as disclosed in the present application shows great improvement in utility over the prior art. And yet, the Examiner assumes that this is predictable and a natural outcome of protein results. This blanket conclusory statement is against the patent laws. Where Trieu states explicitly that small fragment Apo(a) gene does not result in reduction of growth of tumor, the Examiner states that it would require only routine optimization to move forward with gene therapy and expect successful results. This logic does not make sense. Where Trieu states that small fragments of Apo(a) gene fail at reducing tumor growth, a person of skill in the art would be dissuaded from using the same or similar small fragment of gene encoding Apo(a), not be encouraged by it. Although Chang

discloses successful results of using same or similar small fragment of Apo(a) protein, given the overall sense at the time of the invention where gene therapy was not recognized to be an automatically successful and predictable, the presently claimed invention is not obvious over the cited references.

Double Patenting Rejection

Claims 1-4 and 8-10 have been rejected under the obviousness-type double patenting as being unpatentable over claims 3-4, and 11-12 of Patent No. 6,743,428. It is believed that as discussed during the interview held on July 9, 2010, once the presently claimed invention overcomes the obviousness rejection under 35 U.S.C. 103(a) over Chang, the present double patenting rejection also would be overcome because of the relatedness of both of these rejections over Chang.

Conclusion

It is believed that the application is now in condition for allowance. Applicants request the Examiner to issue a notice of Allowance in due course. The Examiner is encouraged to contact the undersigned to further the prosecution of the present invention.

The Commissioner is hereby authorized to charge JHK Law's Deposit Account No. **502486** for such fees required under 37 CFR §§ 1.16 and 1.17 and to credit any overpayment to said Deposit Account No. **502486**.

Respectfully submitted,

JHK Law

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